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Agonist and antagonist activities of the leukotriene analogue BAY u9773 in guinea pig lung parenchyma

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Abstract

BAY u9773(6(*R*)-(4′-carboxyphenylthio)-5(*S*)-hydroxy-7(*E*),9(*E*),11(*Z*),14(*Z*)-eicosatetraenoic acid) is a leukotriene E_4 analogue used to define 'atypical' receptors for cysteinyl–leukotrienes. The aims of this study were first to characterise the intrinsic properties of BAY u9773 in guinea-pig lung parenchyma in vitro and second to study the influence of BAY u9773 on the concentration–response relation for leukotriene D_4 in the same preparation. BAY u9773 in itself caused a concentration-dependent contraction, which was not inhibited by the cyclooxygenase inhibitor indomethacin nor by the 5-lipoxygenase inhibitor zileuton (*N*-(1-benzo-(12)-thien-2-ylethyl)-*N*-hydroxyurea). The *CysLT*$_1$ receptor antagonist ICI 198,615 {(1-((2-methoxy-4-(((phenylsulfonyl)amino) carbonyl)phenyl)methyl)-1*H*-indazol-6-yl)carbamic acid cyclopentyl ester} alone blocked the contractile response to BAY u9773 1 μM, whereas a combination of the TP receptor antagonist BAY u3405 ((3*R*)-3-(4-fluorophenylsulfonamido)-1,2,3,4-tetrahydro-9-carbazolepropanoic acid) and ICI 198,615 was required to block the contraction induced by BAY u9773 10 μM. Together the findings suggest that BAY u9773 acted as a *CysLT*$_1$ receptor agonist and in the higher concentration also as a TP receptor agonist. The *CysLT*$_1$ receptor antagonist ICI 198,615 partly inhibited the contractile response to leukotriene D_4. Pretreatment with BAY u9773 or leukotriene D_4, caused concentration-dependent rightward displacement of the concentration–response curve for leukotriene D_4. The inhibition by BAY u9773 was partial, and not greater than that produced by ICI 198,615. Combination of BAY u9773 and ICI 198,615 did not produce additive inhibition, suggesting that the major part of the leukotriene D_4 induced contraction in guinea pig lung parenchyma is mediated by a *CysLT* receptor with properties distinct from those of previously described *CysLT*$_1$ and *CysLT*$_2$ receptors.

Keywords: Leukotriene receptor; Lung parenchyma; BAY u9773; Leukotriene D_4; ICI 198,615; Thromboxane receptor

1. Introduction

Cysteinyl–leukotrienes (CysLTs, LTC_4, D_4 and E_4) are potent spasmogenic substances in guinea pig airways as well as in other tissues (Drazen et al., 1980; Hedqvist et al., 1980; Piper and Samhoun, 1981; Dahlén et al., 1983). They have been documented to mediate antigen induced contractions of guinea pig lung parenchyma (Steward et al., 1984; Burka, 1985; Wikström Jonsson and Dahlén, 1994), and IgE (Immunoglobulin E)-dependent contractions of human bronchi (Björck and Dahlén, 1993). Bronchoprovocation studies have confirmed that leukotrienes mediate the predominant component of allergen-induced airway obstruction in asthmatics (O'Byrne, 1997). Moreover, leukotriene synthesis inhibitors and leukotriene receptor antagonists have recently been documented to be beneficial in asthma therapy (O'Byrne, 1997).

Despite this striking progress in clinical science, the knowledge about the properties of the receptors for cysteinyl–leukotrienes (*CysLT* receptors) remains incomplete. With no structural and molecular information being available about the cysteinyl–leukotriene receptors, functional studies are required to extend knowledge about atypical receptors for cysteinyl–leukotrienes. On the basis of such functional data, two distinct receptors have been defined for cysteinyl–leukotrienes (Fleisch et al., 1982; Krell et al., 1983; Cuthbert et al., 1989; Labat et al., 1992). Selective antagonists are up to date available only for one subtype, designated the *CysLT*$_1$ receptor (Coleman et al., 1995). These antagonists generally inhibit the action of leukotriene D_4 and leukotriene E_4 in the guinea pig trachea or ileum (Krell et al., 1983; Snyder and Krell, 1984; Gardiner et al., 1990) and the effects of all cysteinyl–leukotrienes in hu-

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man bronchi (Dahlén et al., 1980; Jones et al., 1982; Buckner et al., 1986; Buckner et al., 1990).

There are however several effects of cysteinyl–leukotrienes in man or in human tissues which are not blocked by the current class of antagonists (Labat et al., 1992; Dahlén et al., 1994). The receptors mediating such responses to cysteinyl–leukotrienes have been designated $CysLT_2$ receptors (Coleman et al., 1995). It was discovered that the leukotriene E_4-analogue BAY u9773 (6(*R*)-(4′-carboxyphenylthio)-5(*S*)-hydroxy-7(*E*),9(*E*),11(*Z*),14(*Z*)-eicosatetraenoic acid) had broader antagonistic activity than other antagonists of cysteinyl–leukotrienes, antagonising contractions induced by leukotriene C_4 and leukotriene D_4 in for example human pulmonary veins (Labat et al., 1992), guinea pig trachea, rat lung, ferret spleen and sheep bronchus (Tudhope et al., 1994). We recently established that BAY u9773 was a competitive antagonist of leukotriene C_4 and leukotriene D_4 in sheep trachealis muscle (Wikström Jonsson, 1997) and of leukotriene C_4 in guinea pig ileum longitudinal muscle (Bäck et al., 1996). The compound BAY u9773 is therefore currently the only pharmacological tool available to support the presence of purported $CysLT_2$ receptors.

The guinea pig lung parenchymal strip is among the most sensitive smooth muscle preparations with respect to contractile effects of cysteinyl–leukotrienes (Drazen et al., 1980; Piper and Samhoun, 1981; Sirois et al., 1981; Dahlén et al., 1983). However, it has not been established via which receptor(s) the contraction is evoked. One of the most potent $CysLT_1$ receptor antagonists, ICI 198,615, was reported to competitively antagonise the effect of leukotriene D_4 in guinea pig lung parenchyma with a pA_2 value of 9.5 (Snyder et al., 1987). Subsequent studies have failed to confirm that ICI 198,615 or other potent $CysLT_1$ antagonists are particularly effective antagonists of leukotriene D_4 in guinea pig lung parenchyma (Cuthbert et al., 1989; Tudhope et al., 1994). This uncertainty with regard to the influence of conventional antagonists on the response to leukotriene D_4 in guinea pig lung parenchyma motivated us to test whether BAY u9773 affected the contractile response to leukotriene D_4 differently. A preliminary set of observations (Tudhope et al., 1994) suggested that BAY u9773 did not antagonise contractions induced by cysteinyl–leukotrienes in guinea pig lung parenchyma. This would imply that there exist receptors for cysteinyl–leukotrienes in guinea pig lung parenchyma which cannot be described as either $CysLT_1$ or $CysLT_2$ receptors. It was therefore a main objective with this study to compare the influence of ICI 198,615 and BAY u9773 on the response to leukotriene D_4 in guinea pig lung parenchyma.

Furthermore, BAY u9773 was observed to possess contractile activity in guinea pig lung parenchyma (Tudhope et al., 1994). Contractile properties of BAY u9773 have also been reported in human pulmonary veins (Labat et al., 1992), but were not observed in preparations such as tracheae from guinea pig (Tudhope et al., 1994) or sheep (Wikström Jonsson, 1997) or guinea pig ileum (Bäck et al., 1996). The contractile activity of BAY u9773 in guinea pig lung parenchyma (Tudhope et al., 1994) was described to be indomethacin-sensitive, suggesting that BAY u9773 evoked release of spasmogenic prostanoids from the lung. We felt that these properties might interfere with, or be related to, the *CysLT* antagonism of the compound. Before studying the influence of BAY u9773 on the concentration–response relation to leukotriene D_4, the mechanisms involved in the contraction induced by BAY u9773 were therefore characterised in guinea pig lung parenchyma. Since thromboxane A_2 is the major cyclooxygenase-derived spasmogen in guinea pig lung (Hamberg et al., 1976), the effect of the TP receptor antagonist BAY u3405 on the response to BAY u9773 was compared with that of indomethacin. Likewise, because BAY u9773 is a leukotriene analogue, the influence of the selective $CysLT_1$ receptor antagonist ICI 198,615 on the response to BAY u9773 was also evaluated.

2. Materials and methods

2.1. Lung parenchymal strips

Guinea-pigs (250–550 g body weight) of either sex were sacrificed by cervical dislocation and exsanguination. Following perfusion with ice-cold Tyrode's solution (20 ml) via the pulmonary artery, the lungs were excised and the parenchyma was cut parallel to the peripheral margins yielding 7–8 strips, each having a cross-sectional area of approximately 10 mm^2, a length of about 25 mm and a weight of about 50 mg. The study was approved by the regional committee on animal experimentation ethics.

2.2. Organ bath experiments

The preparations were suspended under a resting tension of 2.5 mN in 5 ml organ baths filled with Tyrode's solution (prepared each day, containing NaCl 149.2 mM, KCl 2.7 mM, $NaHCO_3$ 11.9 mM, glucose 5.5 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 0.5 mM, NaH_2PO_4 0.4 mM). The bath fluid was maintained at 37°C and both the stock bottles of Tyrode's solution and the organ baths were gassed with 6.5% CO_2 in O_2 to keep a pH of 7.4. Contractile responses were studied under non-flow conditions, and registered on Grass model 7 Polygraphs or a Riken Denshi speedex recorder SP-K4 via isometric Grass (Quincy, MA, USA) FT03C or Experimetria (Budapest, Hungary) FSG-01 force-displacement transducers.

Drugs were added to the bath fluid to obtain the indicated final concentrations and vehicle controls were performed for all employed solvents. After an equilibration period of 60 min, a cumulative challenge with histamine

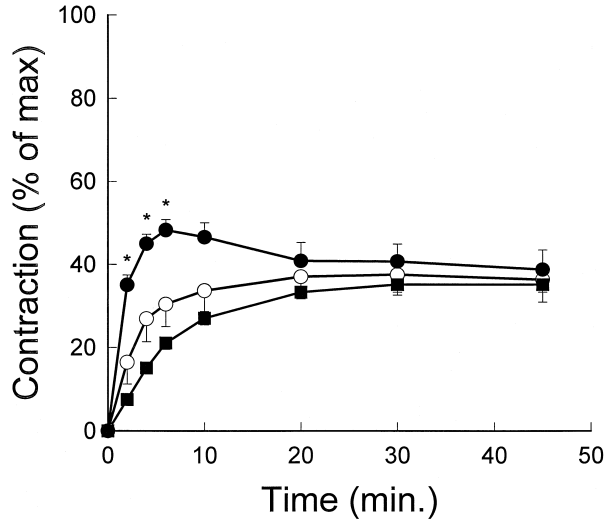

Fig. 1. Time-course of contractions of guinea-pig lung parenchyma evoked by the *CysLT* receptor antagonist BAY u9773 (1 μM, left panel; controls, $n = 8$, open circles, indomethacin 10 μM, $n = 7$, filled circles and zileuton 10 μM, $n = 11$, filled squares). Results are the mean values from the indicated number of experiments (n) and the vertical bars represent the S.E.M. Error bars are omitted when less than symbol size. The response to each concentration of agonist is expressed as a percentage of the maximal response. $* P < 0.05$, values are mean ± S.E.M., Statistical analysis was performed using Student's unpaired, two-tailed *t*-test.

was performed. Histamine was administered again (single dose, 3 μM) repeatedly after 60 min of washing, in order to check tissue reactivity. *S*-hexyl GSH and L-cysteine were present in the baths in all experiments during 15 min before the addition of cysteinyl–leukotrienes or BAY u9773. All experiments with BAY u9773 and leukotriene D_4 were performed in the presence of *S*-hexyl GSH (100 μM) and L-cysteine (5 mM) in order to inhibit metabolism of the leukotrienes (Bäck et al., 1996). All other inhibitors and antagonists were added 10–30 min before addition of BAY u9773. Indomethacin (10 μM) and BAY u3405 (3 μM) were also included in the experiments where concentration–response relations for leukotriene D_4 were studied. Except in the time-course studies, the contractile response to BAY u9773 was evaluated on the plateau of the contraction, 45 min after addition of the compound.

2.3. Drugs and chemicals

Leukotriene C_4 ((5*S*)-hydroxy(6*R*)-*S*-glutathionyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid), Leukotriene D_4 ((5*S*)-hydroxy (6*R*)-*S*-cysteinylglycyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid) and leukotriene E_4 ((5*S*)-hydroxy(6*R*)-*S*-cysteinyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid) were obtained from Merck Frosst (Montreal, Canada) and Cascade Biochemical (Reading, UK). Stock solutions in ethanol or ethanol:water (8:1–3:1, depending on the concentration of leukotrienes) were kept at −70°C.

BAY u9773 (6(*R*)-(4′-carboxyphenylthio)-5(*S*)-hydroxy-7(*E*),9(*E*),11(*Z*),14(*Z*)-eicosatetraenoic acid) (Tudhope et al., 1994) was purchased from Cascade Biochemical (Reading, UK) and stored in ethanol or DMSO (dimethylsulphoxide) under argon at −70°C. The concentration and purity of cysteinyl–leukotrienes and of BAY u9773 were checked by UV-spectrometry, using ε of 45 000 and 40 000, respectively. All other drugs were prepared as fresh solutions each day. ICI 198,615 {(1-((2-methoxy-4-(((phenylsulfonyl)amino) carbonyl)phenyl)methyl)-1*H*-indazol-6-yl)carbamic acid cyclopentyl ester} (Snyder et al., 1987; Aharony et al., 1988) was a gift from Zeneca Pharmaceuticals (Alderley, UK) and was dissolved in DMSO. Indomethacin from Merck, Sharp and Dohme (Rahway, NJ, USA), was dissolved in ethanol, Tris buffer and saline. BAY u3405 ((3*R*)-3-(4-Fluorophenylsulfonamido)-1,2,3,4-tetrahydro-9-carbazolepropanoic acid) (Rosentreter et al., 1989) from Bayer, Leverkusen, Germany was dissolved in ethanol. Zileuton (*N*-(1-benzo-(12)-thien-2-ylethyl)-*N*-hydroxyurea) (Carter et al., 1991) was supplied by Abbot (Chicago, IL, USA) and dissolved in DMSO. Histamine and acetylcholine dissolved in saline and *S*-hexyl glutathione (*S*-hexyl GSH), dissolved in Tris buffer, ethanol and distilled water were all purchased as salts from Sigma Chemical (St. Louis, MO, USA).

2.4. Calculations and statistical methods

The lung of each animal yielded 7–8 parenchymal strips and n equals the number of parenchymal strips studied. Each experimental day, the preparations were randomly assigned to control- and drug-treated groups. All

Table 1
Effects of BAY u3405 and ICI 198,615 on contractions evoked by histamine

Group (n)	Contractile responses to histamine, mean ± S.E.M. (percentage of maximal contraction)					Maximal contraction[a] (mN)
	0.3 μM	1 μM	3 μM	10 μM	30 μM	
Controls (8)	15.4 ± 1.3	26.0 ± 1.9	38.1 ± 2.4	51.3 ± 2.6	64.1 ± 3.7	1.8 ± 0.1
BAY u3405 3 μM (6)	10.3 ± 1.7	21.1 ± 3.1	31.3 ± 4.0	43.8 ± 4.3	55.7 ± 4.1	1.8 ± 0.1
ICI 198,615 1 μM (4)	13.0 ± 5.1	25.3 ± 7.6	37.3 ± 9.6	51.3 ± 13.5	64.0 ± 13.4	1.7 ± 0.3
BAY u3405 3 μM + ICI 198,615 1 μM (7)	17.0 ± 1.8	28.6 ± 2.2	40.9 ± 3.0	54.9 ± 3.7	64.6 ± 4.2	2.0 ± 0.1

None of the groups differed significantly from the control group.
[a]Maximal contractions were determined by addition of 100 μM histamine, 100 μM acetylcholine chloride and 40 mM KCl at the end of the experiment.

responses were expressed in percent of the terminal maximal contraction induced by adding histamine 100 μM, acetylcholine chloride 100 μM and KCl 40 mM at the end of the experiment. Preparations with maximal responses lower than 1 mN were excluded from the study. Concentration–response curves were obtained in the absence and presence of different concentrations of antagonists (Arunlakshana and Schild, 1959). Logarithms of EC_{25}-values were calculated for the mean curve of each group, using the equation of the straight line connecting the two points surrounding the EC_{25} in the concentration-response curve. The concentration ratio (Jenkinson et al., 1995) was then calculated at the EC_{25} level and shifts of the curves using different concentrations of antagonist were estimated and expressed as pK_B. For statistical analysis, Student's unpaired, two-tailed *t*-test was applied. For statistical evaluation, the PC-program SPSS for Windows (SPSS Scandinavia, Sollentuna, Sweden) was used. Contractions are generally expressed in percent of maximal contractions, mean ± S.E.M. Differences were considered as significant when $P < 0.05$.

3. Results

The leukotriene E_4 analogue BAY u9773 (1 μM, Fig. 1) caused a contraction of guinea pig lung parenchyma. The response was slow in onset and had a plateau phase with a duration of more than 45 min. The response evoked by BAY u9773 was neither inhibited by the cyclooxygenase inhibitor indomethacin (10 μM, Fig. 1) nor by the 5-lipoxygenase inhibitor zileuton (10 μM, Fig. 1). In fact, indomethacin significantly potentiated the initial part of the time-course (Fig. 1).

The TP receptor antagonist BAY u3405 (3 μM) did not affect responses evoked by histamine 0.3–100 μM (Table 1), leukotriene D_4 (Table 2) or contractions elicited by the lower concentration of BAY u9773 (1 μM, Fig. 2). However, BAY u3405 significantly depressed the contractile response evoked by the higher concentration of BAY u9773 (10 μM, Fig. 2), whereas indomethacin did not (10 μM, Fig. 2).

The *CysLT*$_1$ receptor antagonist ICI 198,615 (1 μM) abolished the contractile response to the lower concentration of BAY u9773 (1 μM, Fig. 2), without affecting responses to histamine 0.3–100 μM (Table 1). When tested against the higher concentration of BAY u9773, ICI 198,615 also inhibited this contraction (Fig. 2). The remaining contraction was however only blocked when ICI 198,615 and BAY u3405 were combined (Fig. 2). This combination of drugs did not affect the concentration–response relation for histamine 0.3–100 μM or maximal tissue contractility (Table 1).

The influence of pretreatment with BAY u9773 (10 μM) on the contractile response to leukotriene D_4 was evaluated when the spasmogenic actions of BAY u9773

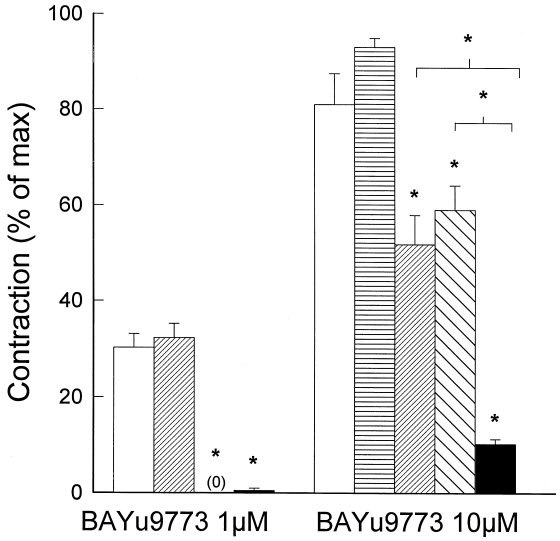

Fig. 2. Effect of *CysLT*$_1$ and TP receptor antagonists on the contraction evoked by BAY u9773 in guinea pig lung parenchyma. Contraction to BAY u9773 1 μM, left, in controls (open bars, $n = 4$) and preparations pretreated with BAY u3405 (3 μM, shaded bar, $n = 4$), ICI 198,615 (1 μM, diagonally striped bar, $n = 4$) and a combination of BAY u3405 and ICI 198,615 (filled bar, $n = 4$). Contractions evoked by BAY u9773 10 μM, right, in controls (open bars, $n = 6$) and preparations pretreated with Indomethacin (10 μM, horizontally striped bar, $n = 3$), BAY u3405 (3μM, shaded bar, $n = 4$), ICI 198,615 (1 μM, diagonally striped bar, $n = 4$) and a combination of BAY u3405 and ICI 198,615 (filled bar, $n = 4$). Results are the mean values from the indicated number of experiments (n) and the vertical bars represent the S.E.M. The response to each concentration of agonist is expressed as a percentage of the maximal response. $* P < 0.05$, values are mean ± S.E.M. Statistical analysis was performed using Student's unpaired, two-tailed *t*-test.

Table 2
Effect of the TP-receptor antagonist BAY u3405 on contractions evoked by leukotriene D_4

Group (n)	Contractile responses to increasing concentrations of leukotriene D_4, mean ± S.E.M. (percent of maximal contraction)					Maximal contraction[a] (mN)
	1 nM	3 nM	10 nM	30 nM	100 nM	
Controls (8)	7.8 ± 1.3	12.1 ± 1.4	27.0 ± 1.9	38.8 ± 2.5	62.9 ± 2.8	1.8 ± 0.1
BAY u3405 3 μM (6)	7.2 ± 1.7	12.4 ± 1.8	32.2 ± 2.2	42.0 ± 1.7	66.3 ± 1.6	1.8 ± 0.1

None of the groups differed significantly from the control group.
[a]Maximal contractions were determined by addition of 100 μM histamine, 100 μM acetylcholine chloride and 40 mM KCl at the end of the experiment.

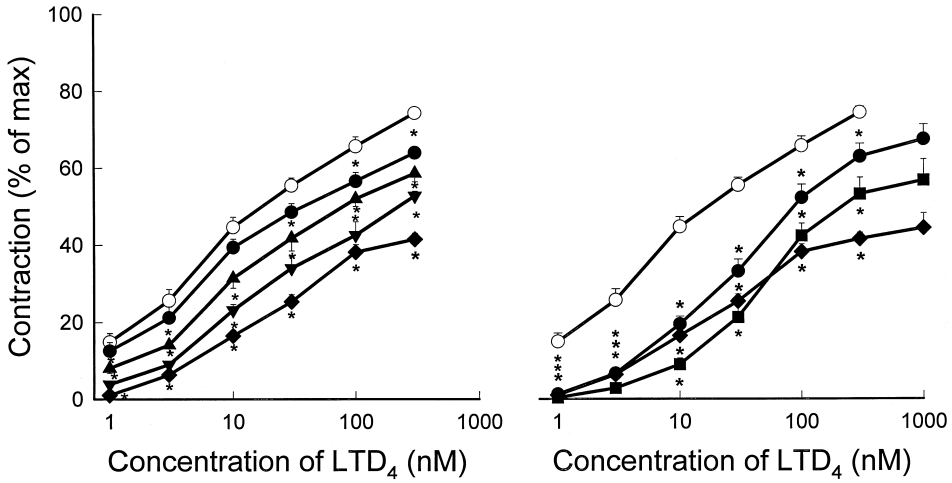

Fig. 3. Influence of different agonists and antagonists on the contractile response to LTD_4 in guinea pig lung parenchyma in the presence of indomethacin (10 μM) and BAY u3405 (3 μM). Left panel: The influence of BAY u9773 (10 μM, filled diamonds, $n = 6$), LTD_4 (1 nM, filled triangles pointing upwards, $n = 5$; 3 nM, filled triangles pointing downwards, $n = 5$) and LTE_4 (3 nM, filled circles, $n = 5$) on the contractile response to LTD_4 (controls seen in open circles, $n = 9$). Right panel: Effect of ICI 198,615 (1 μM, filled circles, $n = 10$), BAY u9773 (10 μM, filled diamonds, $n = 6$) and both antagonists combined (filled squares, $n = 7$) on the contractile concentration–response curve LTD_4 in guinea-pig lung parenchyma (controls seen as open circles, $n = 9$). Results are the mean values from the indicated number of experiments (n) and the vertical bars represent the S.E.M. Error bars are omitted when within symbol size. The response to each concentration of agonist is expressed as a percentage of the maximal response. For clarity, the precontraction was omitted in the graphs and the data was separated into two parts (left and right panel) with the same control group shown in both. $* P < 0.05$. Statistical analysis was performed using Student's unpaired, two-tailed t-test.

were inhibited by BAY u3405 (3 μM, Fig. 3, left panel). Exposure to BAY u9773 under these conditions yielded a precontraction of 27 ± 1.8% of maximum (mean ± S.E.M., $n = 6$). Since BAY u9773 is a leukotriene E_4 analogue, the effect of BAY u9773 on the contractile response to leukotriene D_4 was compared with that of leukotriene E_4. Under the same experimental conditions, leukotriene E_4 (3 nM) also caused a precontraction (13 ± 2.8% of maximum, $n = 5$). Whereas BAY u9773 clearly inhibited the contractile response to leukotriene D_4, leukotriene E_4 only inhibited contractions evoked by the higher concentrations of leukotriene D_4 (Fig. 3, left panel). Pretreatment with leukotriene D_4 1 nM ($n = 5$) and 3 nM ($n = 5$) caused precontractions of 15 ± 3.2 and 24 ± 3.5% of maximum, respectively. Pretreatment with leukotriene D_4 in these concentrations significantly inhibited the response to leukotriene D_4 itself (Fig. 3, left panel). The magnitude of the precontraction caused by 3 nM leukotriene D_4 was not

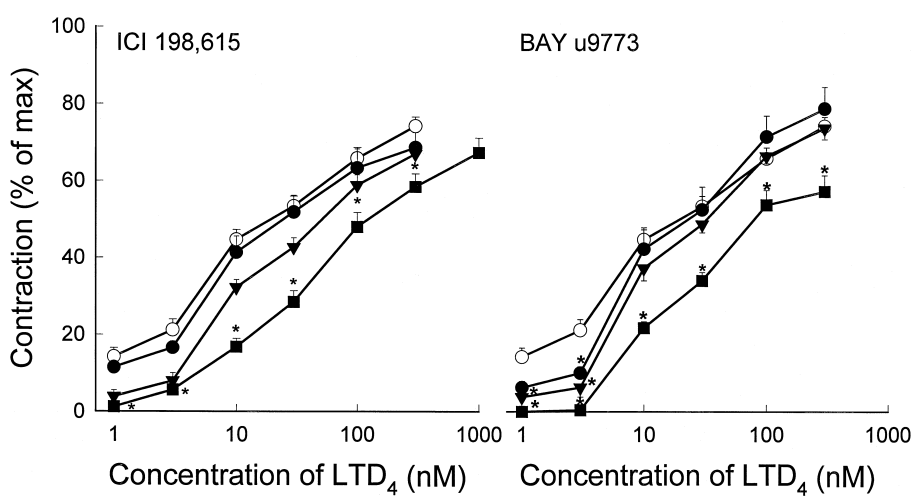

Fig. 4. Concentration–response relations of LTD_4 (controls open circles, $n = 13$) in guinea pig lung parenchyma in the presence of ICI 198,615 (left panel, 10 nM filled circles, $n = 6$, 100 nM filled triangles, $n = 6$, 1000 nM filled squares, $n = 7$) and BAY u9773 (right panel, 10 nM filled circles, $n = 4$, 100 nM filled triangles, $n = 4$ and 1000 nM filled squares, $n = 4$). Results are the mean values from the indicated number of experiments (n) and the vertical bars represent the S.E.M. The response to each concentration of agonist is expressed as a percentage of the maximal response.

significantly different from that caused by BAY u9773 10 μM, whereas the precontractions caused by leukotriene E_4 or 1 nM leukotriene D_4 were significantly smaller.

The *CysLT*$_1$ receptor antagonist ICI 198,615 (1 μM), as well as BAY u9773 inhibited the contractile response elicited by leukotriene D_4 (Fig. 3, right panel). There was no further inhibition of the contractile response to leukotriene D_4 when BAY u9773 (10 μM) was combined with ICI 198,615 (1 μM, Fig. 3, right panel). The inhibition caused by combination of the two drugs was of the same magnitude as that achieved with either compound alone (Fig. 3, right panel).

It was established that the *CysLT*$_1$ receptor antagonist ICI 198,615 and the leukotriene E_4 analogue BAY u9773 caused an apparently concentration-dependent inhibition of the concentration–response relation for leukotriene D_4 (Fig. 4). The inhibition was however mainly significant for the highest concentration of either antagonist. The shifts implicated pK_B values of 7.2 for ICI 198,615 and 6.8 for BAY u9773. The limited availability of BAY u9773 precluded experiments at higher antagonist concentrations.

4. Discussion

The leukotriene E_4 analogue BAY u9773 has provided an important step forward in the characterisation of receptors for cysteinyl–leukotrienes. First, it has been documented to be a competitive, although not very potent, *CysLT*$_1$ receptor antagonist (Tudhope et al., 1994). Second, it has been shown to have a broader antagonistic activity than any other described *CysLT*$_1$ receptor antagonist, also exhibiting competitive antagonism of some responses resistant to published *CysLT*$_1$ receptor antagonists (Labat et al., 1992; Tudhope et al., 1994; Bäck et al., 1996; Wikström Jonsson, 1997). Therefore, sensitivity to BAY u9773 has so far been used as the main criterion to define the *CysLT*$_2$ subclass of receptors for cysteinyl–leukotrienes (Coleman et al., 1995).

Guinea pig lung parenchyma is one of the tissues that is most sensitive to cysteinyl–leukotrienes (Drazen et al., 1980; Piper and Samhoun, 1981; Sirois et al., 1981; Dahlén et al., 1983), but current *CysLT*$_1$ receptor antagonists such as ICI 198,615 have shown variable inhibitory efficacy on cysteinyl–leukotriene responses in this preparation (Snyder et al., 1987; Cuthbert et al., 1989; Tudhope et al., 1994). It was previously reported that BAY u9773 failed to inhibit contractions induced by leukotriene C_4 and leukotriene D_4 in guinea-pig lung parenchyma (Tudhope et al., 1994), adding further confusion to the classification of the cysteinyl–leukotriene receptors in this particular preparation. In fact, a contractile response to BAY u9773 itself was also observed in a limited series of experiments ($n = 4$) (Tudhope et al., 1994).

In the present study, the mechanism of the contraction evoked by BAY u9773 in guinea-pig lung parenchyma was explored and unexpectedly found to involve mechanisms suggesting direct agonism of BAY u9773 at two defined receptors. The contractions to BAY u9773 were sensitive to antagonism by ICI 198,615, the prototype for *CysLT*$_1$ receptor antagonists. Contractions evoked by higher concentrations of BAY u9773 (10 μM) were also sensitive to TP receptor antagonism by BAY u3405. When the TP- and *CysLT*$_1$ receptor antagonists were combined, the contractile effects of even maximal responses to BAY u9773 were almost abolished. The combination of drugs however had no apparent unspecific effects on the preparations, as shown by unchanged reactivity to histamine and no change of maximal contractility.

On the other hand, the contraction evoked by BAY u9773 was not inhibited by cyclooxygenase inhibition, indicating that endogenous formation of prostaglandins or thromboxane did not account for the contraction. In fact, indomethacin caused potentiation of the early part of the contraction, suggesting a possible role for inhibitory cyclooxygenase products.

Neither did the *CysLT*$_1$ agonism seem to be explained by induction of endogenous release of 5-lipoxygenase products, because the 5-lipoxygenase inhibitor zileuton did not modify the response to BAY u9773. Our findings support that BAY u9773 is a combined *CysLT*$_1$ and TP-receptor agonist, expressing TP receptor agonism in a higher concentration interval. The latter finding was intriguing, since BAY u9773 has no agonist activity on guinea-pig trachea, human bronchial muscle or human lung strip (Tudhope et al., 1994), which all contain TP receptors. One possibility is that guinea-pig lung parenchyma has a different type of TP receptor than other tissues. However, previous data have supported that prostanoid contractile agonists act through TP receptors in guinea pig as well as human lung parenchyma, whereas they act through TP- and EP_1-receptors in guinea-pig trachea (McKenniff et al., 1988). Furthermore, non-cyclooxygenase derived TP receptor agonists such as 8-epi-$PGF_{2\alpha}$ or related *iso*-prostanes have also been described (Morrow et al., 1992). Therefore, another possibility is that BAY u9773 stimulates release of such compounds.

Nevertheless, when the contractile activity of BAY u9773 was attenuated by the TP receptor antagonist BAY u3405, BAY u9773 caused a significant inhibition of the contractile response to leukotriene D_4. The concentration of BAY u3405 used completely blocked the TP receptors in this preparation (Wikström Jonsson and Dahlén, 1994). The antagonism of leukotriene D_4 by BAY u9773 in the presence of the TP receptor antagonist BAY u3405 thus makes it unlikely that the previously mentioned TP receptor agonism was the mechanism of its inhibition of leukotriene D_4. Furthermore, antagonism of the response to leukotriene D_4 occurred also with the lower concentrations of BAY u9773 which were found not to agonise TP receptors, although this was significant only for low concentrations of leukotriene D_4. The conclusion that the

inhibition of the contractile response to leukotriene D_4 caused by BAY u9773 was not due to its property of being an agonist at TP receptors gets circumstantial support from data obtained in guinea-pig ileum, where pre-treatment with the TP receptor agonist U-46,619 did not alter the response to leukotriene C_4 (Bäck et al., 1996).

The well characterised and specific *CysLT*$_1$ receptor antagonist ICI 198,615 (Snyder et al., 1987) also inhibited the contractile response to leukotriene D_4 in guinea-pig lung parenchyma, yielding a pK_B value of 7.2, not significantly different from that established for BAY u9773. The pK_B value obtained with BAY u9773 (6.8) is similar to the pA_2 values obtained in other preparations (Tudhope et al., 1994; Bäck et al., 1996; Wikström Jonsson, 1997). In the present study, complete Schild-plots were not constructed, since the amounts of BAY u9773 required for obtaining concentrations over 10 μM in the organ baths were not available. These findings, together with the lack of additive effects when combining the two antagonists, suggest that both ICI 198,615 and BAY u9773 inhibited the response to leukotriene D_4 via the same mechanism, namely antagonism at *CysLT*$_1$ receptors, supporting the existence of *CysLT*$_1$ receptors in guinea-pig lung parenchyma.

The mechanism for antagonism of leukotriene D_4 by BAY u9773 in the presence of the TP receptor antagonist BAY u3405 may alternatively be explained by the *CysLT*$_1$ receptor agonism we observed with BAY u9773. This hypothesis is supported by the finding that leukotriene D_4 was able to antagonise itself. Furthermore, leukotriene E_4, an agonist at *CysLT*$_1$ receptors (Buckner et al., 1990; Norman et al., 1994) but a poor *CysLT*$_2$ receptor agonist (Labat et al., 1992; Gardiner et al., 1993; Wikström Jonsson, 1997), also exerted weak antagonism of leukotriene D_4. The agonistic properties of BAY u9773 however create difficulties when interpreting the data. Both leukotriene D_4 (3 nM) and BAY u9773 caused similar degrees of precontraction and leukotriene D_4 antagonism. An agonistic effect of BAY u9773 has also been found in another preparation where BAY u9773 antagonised cysteinyl–leukotriene induced contractions, namely the human pulmonary vein (Labat et al., 1992). Nevertheless, competitive antagonism of cysteinyl–leukotrienes has been observed in several preparations where BAY u9773 had no spasmogenic effects, such as guinea-pig trachea, rat lung and sheep bronchus (Tudhope et al., 1994). Thus, although it remains possible that the inhibition we observed with BAY u9773 in guinea-pig lung parenchyma was related in part to the precontraction itself, the agonistic property of BAY u9773 cannot be the explanation of its ability to antagonise cysteinyl–leukotrienes.

It was previously reported that ICI 198,615 was a rather potent *CysLT*$_1$ receptor antagonist in guinea-pig lung parenchyma, when its effects on the contractile response to leukotriene D_4 was studied without indomethacin pre-treatment (Snyder et al., 1987). In fact, ICI 198,615 was 100 times less potent in this study and in another investigation (Tudhope et al., 1994), both conducted in the presence of indomethacin. This may relate to the observation that the *CysLT*$_1$ antagonistic effects of ICI 198,615 in guinea-pig lung parenchyma were smaller in the presence than in the absence of indomethacin (Norman et al., 1990). Although cysteinyl–leukotriene induced release of the spasmogenic cyclooxygenase product thromboxane A_2 from guinea-pig lung parenchyma is a well-known phenomenon (Folco, 1981; Piper and Samhoun, 1982; Dahlén et al., 1983), it is not clear why inhibition of such presumably post-receptor events should cause an apparent change in the antagonist efficacy.

The results do not provide evidence for the existence of *CysLT*$_2$ receptors in this preparation. First, BAY u9773 did not provide any further antagonism of the contractile response evoked by leukotriene D_4 when combined with ICI 198,615. Second, leukotriene E_4 has previously been shown to antagonise contractions induced by leukotriene D_4 in preparations containing *CysLT*$_2$ receptors such as sheep tracheal smooth muscle (Tomioka et al., 1991), ferret spleen (Gardiner et al., 1993), human pulmonary veins (Labat et al., 1992) and ferret trachea (Snyder and Krell, 1986), but was not particularly effective in guinea-pig lung parenchyma. Therefore, since a large part of the response to leukotriene D_4 in this study could not be inhibited by either *CysLT*$_1$ or *CysLT*$_2$ receptor antagonists, the results indicate the presence of a *CysLT* receptor with properties distinct from those of *CysLT*$_1$ and *CysLT*$_2$ receptors previously described.

Incidentally, since the *CysLT*$_2$ receptor is particularly abundant in guinea-pig trachea (Hay et al., 1987; Snyder et al., 1987; Buckner et al., 1990; Tudhope et al., 1994), the failure to demonstrate a *CysLT*$_2$ receptor mediated response to leukotriene D_4 in this study of the guinea-pig lung parenchyma supports that the proportion of central airway tissue in guinea-pig lung parenchyma is very small, as previously indicated on the basis of morphological examinations (Drazen and Schneider, 1978).

In summary, this study describes a previously unknown feature of BAY u9773, namely dual agonistic activity at *CysLT*$_1$ and TP receptors. Furthermore, we observe antagonism at *CysLT*$_1$ receptors (Tudhope et al., 1994), and our findings support the existence of a population of such receptors in guinea-pig lung parenchyma. We could not confirm existence of *CysLT*$_2$ receptors, as currently defined, in guinea pig lung parenchyma. We therefore contribute new independent support for the hypothesis that cysteinyl–leukotrienes probably act predominantly at a new receptor type in this preparation (Tudhope et al., 1994).

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